








ORIGINAL RESEARCH

Twelve Variants Polygenic Score for Low-Density Lipoprotein Cholesterol Distribution in a Large Cohort of Patients With Clinically Diagnosed Familial Hypercholesterolemia With or Without Causative Mutations

Elena Olmastroni , MSc; Marta Gazzotti , PhD; Marcello Arca , MD; Maurizio Averna , MD; Angela Pirillo , PhD; Alberico Luigi Catapano , PhD; Manuela Casula , PhD; the LIPIGEN Study Group*

BACKGROUND: A significant proportion of individuals clinically diagnosed with familial hypercholesterolemia (FH), but without any disease-causing mutation, are likely to have polygenic hypercholesterolemia. We evaluated the distribution of a polygenic risk score, consisting of 12 low-density lipoprotein cholesterol (LDL-C)-raising variants (polygenic LDL-C risk score), in subjects with a clinical diagnosis of FH.

METHODS AND RESULTS: Within the Lipid Transport Disorders Italian Genetic Network (LIPIGEN) study, 875 patients who were FH-mutation positive (women, 54.75%; mean age, 42.47±15.00 years) and 644 patients who were FH-mutation negative (women, 54.21%; mean age, 49.73±13.54 years) were evaluated. Patients who were FH-mutation negative had lower mean levels of pretreatment LDL-C than patients who were FH-mutation positive (217.14±55.49 versus 270.52±68.59 mg/dL, $P<0.0001$). The mean value (±SD) of the polygenic LDL-C risk score was 1.00 (±0.18) in patients who were FH-mutation negative and 0.94 (±0.20) in patients who were FH-mutation positive ($P<0.0001$). In the receiver operating characteristic analysis, the area under the curve for recognizing subjects characterized by polygenic hypercholesterolemia was 0.59 (95% CI, 0.56–0.62), with sensitivity and specificity being 78% and 36%, respectively, at 0.905 as a cutoff value. Higher mean polygenic LDL-C risk score levels were observed among patients who were FH-mutation negative having pretreatment LDL-C levels in the range of 150 to 350 mg/dL (150–249 mg/dL: 1.01 versus 0.91, $P<0.0001$; 250–349 mg/dL: 1.02 versus 0.95, $P=0.0001$). A positive correlation between polygenic LDL-C risk score and pretreatment LDL-C levels was observed among patients with FH independently of the presence of causative mutations.

CONCLUSIONS: This analysis confirms the role of polymorphisms in modulating LDL-C levels, even in patients with genetically confirmed FH. More data are needed to support the use of the polygenic score in routine clinical practice.

Key Words: familial hypercholesterolemia ■ molecular diagnosis ■ polygenic risk score

Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder characterized by life-long exposure to elevated low-density lipoprotein

cholesterol (LDL-C) levels, with an estimated prevalence as high as 1 in 200 people.^{1,2} Patients with FH are at significantly higher risk of premature coronary disease

Correspondence to: Manuela Casula, PhD, Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan, via Balzaretti 9, 20133 Milan, Italy. E-mail: manuela.casula@unimi.it

*A complete list of the LIPIGEN Study group members can be found in the Appendix at the end of the article.

Supplemental Material for this article is available at <https://www.ahajournals.org/doi/suppl/10.1161/JAHA.121.023668>

For Sources of Funding and Disclosures, see page 9.

© 2022 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- This analysis investigated the impact of a low-density lipoprotein polygenic score on low-density lipoprotein cholesterol levels in a large cohort of subjects with familial hypercholesterolemia with or without a causative mutation.
- Our findings indicate that the polygenic or monogenic causes of hypercholesterolemia are not mutually exclusive, but rather interacting entities.
- Our results led us to hypothesize that variants in multiple low-density lipoprotein cholesterol-raising genes can play a role in determining low-density lipoprotein cholesterol levels even in patients with monogenic familial hypercholesterolemia.

What Are the Clinical Implications?

- These data can integrate the evidence on polygenic scores associated with hypercholesterolemia, deepening the knowledge about the genetic basis of this pathology.
- Our results undermine the clinical benefit of the evaluation of the low-density lipoprotein polygenic score to guide diagnosis.
- More data are needed to investigate the association between the polygenic score and the risk of atherosclerotic cardiovascular disease to support the use of the score in clinical practice to accurately assess the risk.

Nonstandard Abbreviations and Acronyms

FH	familial hypercholesterolemia
FH/M+	mutation-positive FH
FH/M-	mutation-negative FH
LDLc-score	polygenic low-density lipoprotein cholesterol risk score

compared with the general population; however, a timely diagnosis and initiation of efficacious LDL-C-lowering therapies can significantly delay the onset of cardiovascular events and even normalize life expectancy.³

The most common FH-causing variants involve mutations in the *LDLR* gene, followed by mutations in the genes encoding the APOB (apolipoprotein B-100) and PCSK9 (proprotein convertase subtilisin/kexin type 9),⁴ or biallelic mutations in the gene encoding for LDLRAP1 (low-density lipoprotein receptor adaptor protein 1).⁵ Several attempts to identify new genes that could account for the FH phenotype⁶ failed to detect single variants exhibiting effects equaling those in the previously mentioned genes, though mutations in

APOE and *LIPA* genes have been reported to play a role.^{7,8} Depending upon the diagnostic criteria used, a causative mutation in candidate genes can be detected in 40% to 80% of clinically defined patients with FH.⁹⁻¹¹ In all the other cases, the cause for the clinical phenotype of hypercholesterolemia remains undefined.

A possible explanation is that, in clinically diagnosed patients with FH without mutations in the classical genes, elevated LDL-C levels might have a polygenic cause. Such patients likely carry a cluster of common polymorphisms affecting several loci associated with raised LDL-C levels. To address this question, polygenic risk scores have been developed to predict LDL-C levels and atherosclerotic cardiovascular disease risk in these individuals.^{12,13} A meta-analysis of genome-wide association studies by the Global Lipid Genetic Consortium identified several loci associated with raised LDL-C levels.^{14,15} Talmud et al demonstrated that individuals carrying multiple LDL-C-raising single nucleotide polymorphisms may present with LDL-C levels similar to those observed in patients carrying FH-causative mutations.¹⁶ In addition, in clinically diagnosed patients with FH without known monogenic mutations as compared with healthy controls, an elevated polygenic LDL-C score, calculated by incorporating 12 LDL-C-raising alleles, was reported, suggesting a potential polygenic cause for the hypercholesterolemic phenotype.¹³ Even in patients with monogenic FH, a polygenic contribution may subsist, likely contributing to the variable phenotypic expression observed in patients carrying the same FH-causative mutation.¹⁷

Despite the growing attention to this aspect, several questions still remain to be addressed. It is not well defined to what extent polygenic hypercholesterolemia contributes to the prevalence of clinically suspected FH. Moreover, there is no consensus about the cutoff value of each polygenic score best discriminating FH from polygenic hypercholesterolemia. Finally, the impact of elevated polygenic score in predicting LDL-C levels in patients with or without monogenic variants has been only marginally investigated. These uncertainties still make it unclear whether and to what extent a polygenic involvement should be investigated in the clinical practice. As a consequence, the clinical potential of polygenic risk scores is still debated.¹⁸

In this study, we aimed at describing the distribution of the polygenic LDL-C risk score proposed by Talmud et al¹³ (LDLc-score) in a large cohort of Italian subjects clinically diagnosed with FH, by comparing this distribution in subjects with or without causative mutations and evaluating its correlation with LDL-C levels.

METHODS

The authors declare that all supporting data are available within the article and its online supplementary files.

This analysis was performed in patients enrolled in the Lipid Transport Disorders Italian Genetic Network (LIPIGEN) study, an observational, multicenter, retrospective, and prospective ongoing study started in 2012 and aimed at identifying and registering patients with FH in Italy.^{19,20}

Detailed information about the procedures of LIPIGEN study has been previously published.^{19,21} In brief, patients with hypercholesterolemia attending >50 lipid clinics throughout Italy were enrolled in the registry if they had a clinical diagnosis of FH. The clinical diagnosis may be based either on the application of the clinical score or on the decision of the lipid specialist, supported by anomalies in the lipid profile or by the presence of a familial history of premature cardiovascular disease. After the visit by a specialized physician, patients with clinically suspicious primary hypercholesterolemia are referred for genetic testing of the appropriate candidate genes. Collected data include demographic and clinical data (age, sex, personal and family history of hypercholesterolemia or premature cardiovascular or cerebrovascular events, data from physical examination), pharmacological therapies, and biochemical data.

In the present analysis, we included adults with a clinical diagnosis of FH and with genetic test performed in a centralized laboratory searching for possible mutations in candidate genes (*LDLR*, *APOB*, *PCSK9*, *APOE*, *LDLRAP1*) and evaluating the 12 common LDL-C-raising single nucleotide polymorphisms included in the LDLc-score¹³ (rs2479409 [*PCSK9* gene], rs629301 [*CELSR2* gene], rs1367117 [*APOB* gene], rs4299376 [*ABCG8* gene], rs1564348 [*SLC22A1* gene], rs1800562 [*HFE* gene], rs3757354 [*MYLIP* gene], rs11220462 [*ST3GAL4* gene], rs8017377 [*NYNRIN* gene], rs6511720 [*LDLR* gene], rs429358 [*APOE* gene], rs7412 [*APOE* gene]).

We selected patients with mutation-positive FH, with at least 1 causative mutation in 1 of the candidate genes (FH/M+), and patients with mutation-negative FH, without mutations in any of these genes²² (FH/M-). Subjects presenting only variants of uncertain significance were excluded from the analysis.²²

The LIPIGEN study has been approved by the Institutional Review Board of each participating center and conducted in accordance with the principles of the Helsinki Declaration, the standards of Good Clinical Practice (ICH GCP), the data protection laws, and other applicable regulations. Patients of any age and sex, with clinical suspicion of familial hypercholesterolaemia, who are able to understand the study procedures and who voluntarily agree to participate by providing written informed consent may be included into the study.

Statistical Analysis

Continuous variables are presented as mean±SD, whereas categorical variables are presented as cases

and percentage rate. To compare mean LDLc-score values between FH/M+ and FH/M- groups, the Student *t* test was applied when the distributions did not fail the assumptions of normality.

A receiver operating characteristic analysis was also performed to determine whether the LDLc-score might discriminate individuals with a causative mutation from subjects who were mutation-negative. Sensitivity and specificity are presented as the measures to assess the effectiveness of the polygenic score, which indicates the ability of the LDLc-score to discriminate FH/M+ from subjects who were FH/M-. The Youden index method was applied to define the optimal cut point, the point maximizing the Youden function, which is the difference between true positive rate and false positive rate over all possible cut point values.

Correlations between the LDLc-score and LDL-C levels in subjects who were FH/M+ and FH/M- were assessed using the Spearman rank coefficient, and the Loess procedure was used to fit a smooth curve to the data, which attempts to capture the general pattern. The correlation was also tested among patients sharing the same mutation, selecting the most frequent one.

All analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC). Statistical significance was set at the 0.05 level for every analysis performed.

RESULTS

A total of 875 patients who were FH/M+ and 644 patients who were FH/M- were identified. Demographic and clinical data of FH/M- and FH/M+ groups are shown in the Table.

Although all patients who were FH/M- had a clinical phenotype consistent with a diagnosis of FH, they had lower mean levels of total cholesterol and pretreatment LDL-C, and higher levels of HDL cholesterol, triglycerides, and lipoprotein(a) than patients in the FH/M+ group (Table). Conversely, there were no statistically significant differences in the clinical history of premature coronary heart disease, or premature cerebral or peripheral vascular disease between individuals who were FH/M- and FH/M+. Instead, the prevalence of tendinous xanthomata and arcus cornealis were significantly higher in the FH/M+ group (17.49% versus 4.19% and 13.71% versus 10.56%, respectively). No significant differences in lipid-lowering therapies were observed.

The distribution of the LDLc-score by genetic diagnosis is reported in Figure 1A. The mean value of the LDLc-score was 1.00 (±0.18) in patients who were FH/M- and 0.94 (±0.20) in patients who were FH/M+ (*P* value for the difference between means<0.0001, Figure 1B). Stratifying by pretreatment LDL-C levels,

Table. Clinical, Demographic, and Biochemical Profile of Adults With and Without an Identified Causative Mutation

	FH/M–, N=644	FH/M+, N=875	P value
	Mean [SD]/median [IQR]*	Mean [SD]/median [IQR]*	
Age at baseline, y	49.73 [13.54]	42.47 [14.96]	<0.0001
Total cholesterol, mg/dL	272.69 [72.63]	313.05 [86.26]	<0.0001
Triglycerides, mg/dL†	126 [89–177]	98 [71–137]	<0.0001
HDL-C, mg/dL	59.88 [17.12]	56.1 [15.07]	<0.0001
Lp(a), mg/dL*‡	39.95 [8.4–98]	19.05 [8.55–37]	0.003
Glucose, mg/dL‡	94.78 [23.78]	89.29 [18.44]	0.0002
Pretreatment LDL-C, mg/dL	217.14 [55.49]	270.52 [68.59]	<0.0001
	N (%)	N (%)	P value
Women	348 (54.21)	478 (54.75)	0.83
First-degree relative with premature CHD	214 (33.23)	352 (40.23)	0.0205
First-degree relative with LDL-C >95th percentile	437 (67.86)	752 (85.94)	<0.0001
First-degree relative with tendinous xanthomata and/or arcus cornealis	19 (2.95)	101 (11.54)	<0.0001
Children <18 years with LDL-C >95th percentile	80 (12.42)	197 (22.51)	<0.0001
Clinical history of premature CHD	50 (7.76)	76 (8.69)	0.52
Clinical history of premature cerebral or peripheral vascular disease	27 (4.19)	35 (4.00)	0.85
Tendinous xanthomata	27 (4.19)	153 (17.49)	<0.0001
Arcus cornealis before age 45 y	68 (10.56)	120 (13.71)	0.07
Pretreatment LDL-C value			
155–190 mg/dL	93 (14.44)	52 (5.94)	<0.0001
191–250 mg/dL	335 (52.02)	308 (35.20)	
251–325 mg/dL	124 (19.25)	322 (36.80)	
>325 mg/dL	20 (3.11)	171 (19.54)	
Lipid-lowering therapy	198 (30.75)	268 (30.63)	0.96

CHD indicates coronary heart disease; FH, familial hypercholesterolemia; FH/M+, patients with mutation-positive FH; FH/M–, patients with mutation-negative FH; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; and Lp(a), lipoprotein(a).

*Median [interquartile range].

†N=124 (FH/M–) and N=172 (FH/M+).

‡N=392 (FH/M–) and N=495 (FH/M+).

higher mean LDLc-score levels (Figure 2) were observed among patients who were FH/M– having LDL-C levels between 150 and 350 mg/dL. However, when the receiver operating characteristic analysis was performed (Figure S1A), the area under the curve predicting polygenic hypercholesterolemia was 0.59 (95% CI, 0.56–0.62), with sensitivity and specificity being 77% and 36%, respectively, at 0.905 as a cutoff value (Figure S1B).

When we explored the correlation between LDLc-score and LDL-C levels in subjects who were FH/M+ or FH/M–, a positive trend was observed among subjects who were FH/M– (R , 0.13; $P=0.0006$) that was even more marked among subjects who were FH/M+ (R , 0.15; $P<0.0001$) (Figure 3). We also confirmed this correlation in a subgroup of patients carrying the most frequent mutation affecting *LDLR* (c.662A>G, p. Asp221Gly [N=72, Figure S2]; R , 0.31; $P=0.009$).

DISCUSSION

In recent years, because of the methodological advances in genetic analysis techniques and to genome-wide association studies, several polygenic scores for LDL-C have been proposed that may help explain the significant proportion of patients having a clinical phenotype of FH but with a negative genetic test for mutations of known causative genes.¹⁸

Summary of Our Results and Comparisons

In the present analysis, the polygenic score proposed by Talmud et al¹³ was tested in a population of adults with a clinical diagnosis of FH. Compared with the original publication, our study population was larger, but with comparable LDL-C levels and mean values of LDLc-score (1.00 in FH/M– and 0.94 FH/M+ in

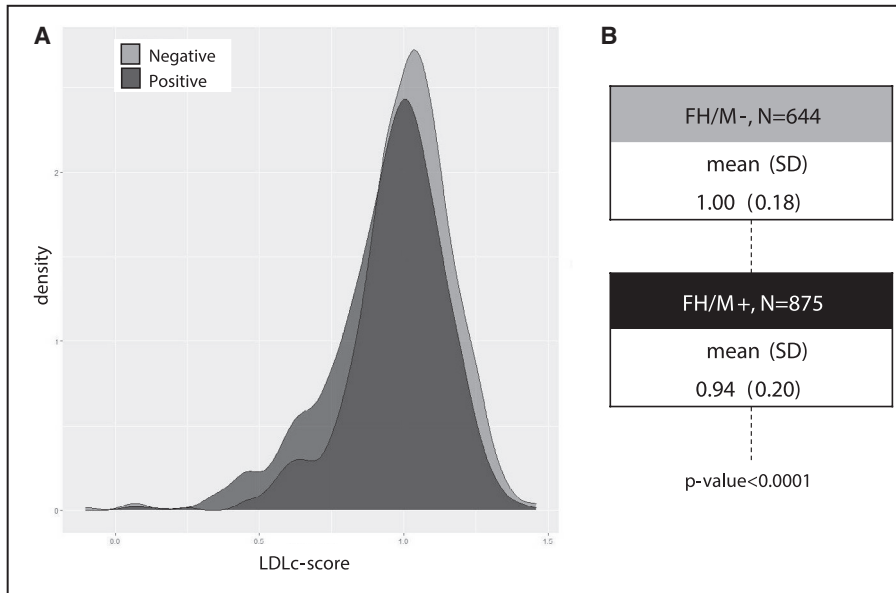


Figure 1. Distribution (A) and mean (SD) values (B) of the LDLc-score in FH/M- and FH/M+ patients with FH.
 FH indicates familial hypercholesterolemia; FH/M+, patients with mutation-positive FH; FH/M-, patients with mutation-negative FH; and LDLc-score, polygenic low-density lipoprotein cholesterol risk score.

the LIPIGEN cohort, and 1.00 and 0.95, respectively, in Talmud et al). These results validate the use of the LDLc-score in a different population of European ancestry.²³

Talmud et al also described the correlation between the LDLc-score and LDL-C concentrations in a healthy group of White men and women from the UK Whitehall II study,¹³ showing increasing levels of

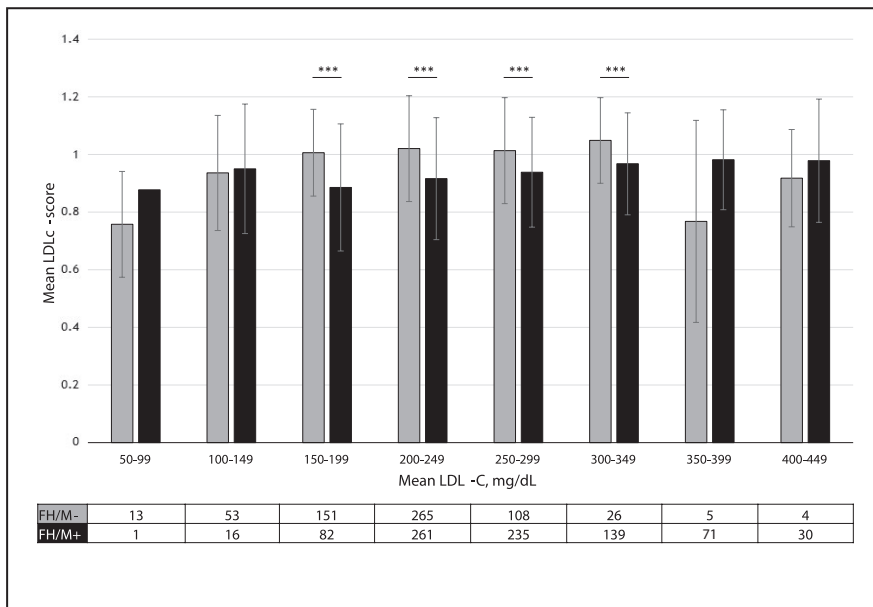


Figure 2. Mean values of LDLc-score by LDL-C classes in patients with FH/M- and FH/M+ FH.
 FH indicates familial hypercholesterolemia; FH/M+, patients with mutation-positive FH; FH/M-, patients with mutation-negative FH; LDL-C, low-density lipoprotein cholesterol; and LDLc-score, polygenic LDL-C risk score. *** means P value for differences among genetic classes lower than 0.001 (P value<0.001).

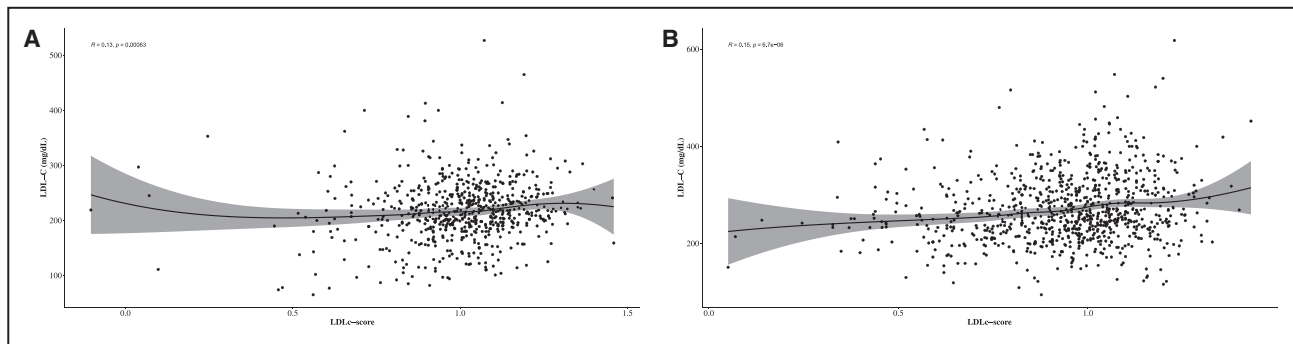


Figure 3. Correlation between LDL-C levels and LDLc-score in mutation-negative (A) and mutation-positive (B) FH patients. FH indicates familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; and LDLc-score, polygenic LDL-C risk score.

LDL-C, ranging from 145 mg/dL to 190 mg/dL, from the first to the last LDLc-score decile (0.43–1.23). In our cohort, we confirmed this correlation both in subjects who were FH/M+ and FH/M–, with LDL-C levels ranging from 182 to 230 mg/dL and from 258 to 281 mg/dL, respectively, from the first to the last LDLc-score decile (Figure S3).

The impact of the polygenic score seems to be more relevant among subjects who were FH/M+, as demonstrated by the Pearson correlation coefficient. The additional impact of the polygenic score in subjects with monogenic FH is also supported by the positive correlation between LDLc-score and LDL-C levels in subjects bearing the same causative mutation (Figure S2). These findings indicated that the polygenic or monogenic causes of hypercholesterolemia are not mutually exclusive, but rather interacting entities.¹⁸ Although in both groups the mean LDL-C levels increased as the score increased, mean LDL-C levels in subjects who were FH/M+ were \approx 30% higher than in subjects who were FH/M– within the same deciles of the LDLc-score.

In addition, our results led us to hypothesize a possible involvement of additional factors (ie, diet, lifestyle habits) in determining the variability of LDL-C levels. In the absence of a known causative mutation, the increase in LDL-C levels would be expected to be driven by polymorphisms in multiple genes included in the LDLc-score. However, the difference in the polygenic score among FH/M– and FH/M+ individuals is modest both in our analysis and in other studies.^{13,24} Moreover, the comparison between LIPIGEN subjects who were H/M– and the healthy subjects from the Whitehall II study showed that the LDL-C level in the former is about 30% higher than in the latter group, despite comparable polygenic score values. These observations suggest that, in subjects with the FH phenotype but lacking a causative mutation (FH/M–), lipid levels may be determined by the interplay between genetic factors, including the polymorphisms evaluated in our

study but also other unknown genetic determinants, and environmental factors.

A multifactorial nature, with the concurrent participation of genetic, epigenetic, and environmental factors, has been suggested for cardiovascular disease²⁵ as well as for many other human disorders such as cancer or diabetes.^{26,27} The exposure to environmental factors can modulate a genetic predisposition, and in turn, genetic predisposition can modify the effects of the environment.^{28,29} The still incomplete understanding of the genetic basis of cardiovascular disease and the crucial impact that genes, environment, and their unceasing interaction exert on this condition can explain, at least in part, the relative failure of lifestyle interventions to prevent and treat cardiovascular disease and its risk factors. This calls for a better understanding of the complex interplay between genomic, environmental, and epigenetic contributions to cardiovascular disease with the aim of improving diagnosis, treatment, and the approach to each patient.³⁰

Clinical Implications

Many studies have addressed the issue of the diagnostic and prognostic usefulness of genetic scores and their clinical potential in the management of patients with hypercholesterolemia.

The rationale is related to the need to identify subjects having a lifetime exposure to high levels of LDL-C, who unveil an increased risk of early cardiovascular events. However, this point calls into question a further aspect that is missing in the current literature, namely, the characterization of the trend of LDL-C levels from youth to adulthood in subjects with polygenic hypercholesterolemia. In these individuals, in whom genetic susceptibility plays a relevant role, additional environmental factors may contribute the increase in LDL-C levels later in life. Thus, another area deserving further research is the possible gene–environment interaction, which implies that the

effect of the polygenic score would be affected by environmental risk factors.³¹

Previous studies have established that polygenic scores for LDL-C independently associate with the risk of CVD. In a study including participants from the UK Biobank cohort, the CV risk increased in a dose-dependent manner with increasing LDL-C polygenic score, with a hazard ratio of 1.35 (95% CI, 1.30–1.40) for the 10th decile compared with the first decile of the polygenic score.³² Therefore, improving current risk models by means of these scores, which allow quantifying the lifelong cumulative burden of LDL-C, might be crucial in clinical practice, because it can improve both diagnosis and long-term prognosis, especially in young subjects, or highlight the long-lasting exposure to high cholesterol levels, which may be relevant in patients diagnosed later in life. This, in turn, may help in defining which patients are likely to benefit most from pharmacological interventions.^{31,33}

However, to translate this evidence into personalized indications for the patients in the clinical practice, some aspects need to be taken into consideration. First, polygenic hypercholesterolemia, unlike monogenic hypercholesterolemia, is not a dichotomous diagnosis but rather a continuous scale that confers cardiovascular disease risk in a dose-dependent manner. Second, whereas a mutation that negatively impacts LDL receptor activity necessarily leads to an increase in circulating LDL-C levels, the polygenic score is indicative of a greater probability of having high LDL-C concentrations. In this context, where the impact of other determinants, such as diet, lifestyle, or comorbidities may be larger, the approach to the patient must be highly personalized. Third, cascade screening and analysis of segregation pattern are crucial in monogenic FH, where only 1 mutation is responsible for the clinical phenotype,^{34–36} but polygenic hypercholesterolemia does not follow an autosomal dominant pattern of inheritance, resulting in a disruption of the cascade screening.

Strengths and Limitations

The major strength of this study is the analysis of an LDL-C polygenic score on a large sample of subjects with a clinical diagnosis of FH. Furthermore, the participation of all specialists in the LIPIGEN network and the centralized laboratory assure a shared approach for the clinical diagnosis and a unique analytical procedure.

We used the score proposed by Talmud et al,¹³ including 12 single nucleotide polymorphisms and based on Global Lipid Genetic Consortium data from >100 000 participants.¹⁴ Even if it is likely an unbiased and robust genetic instrument for LDL-C-raising alleles, we cannot exclude the possibility of further refinements.

Finally, another limitation of the study is that we cannot address the contribution of the LDL-C-score to the development of atherosclerotic cardiovascular disease in terms of future events, because the collection of follow-up data is still ongoing.

CONCLUSIONS

The results of this analysis, which applied a polygenic risk score to a large sample of subjects with a clinical diagnosis of FH, confirmed the role of polymorphisms in modulating LDL-C levels, and suggested that variants in multiple LDL-C-raising genes can play a role in determining LDL-C even in patients with monogenic FH. These data support the application of polygenic risk scores to refine the diagnosis and the prediction of future cardiovascular risk.

APPENDIX

LIPIGEN Study Group

Members of the LIPIGEN Steering Committee: Arca Marcello¹, Averna Maurizio², Bertolini Stefano³, Calandra Sebastiano⁴, Catapano Alberico Luigi⁵, Tarugi Patrizia⁴. Principal Investigators: Coordinator Center: Pellegatta Fabio⁶. Participant Centers: Arca Marcello¹, Averna Maurizio², Bartoli Andrea⁷, Benso Andrea⁸, Biasucci Giacomo⁹, Biolo Gianni¹⁰, Bonanni Luca¹¹, Bonomo Katia¹², Borghi Claudio¹³, Bossi Antonio Carlo¹⁴, Branchi Adriana¹⁵, Calabrò Paolo¹⁶, Carubbi Francesca¹⁷, Cipollone Francesco¹⁸, Citroni Nadia¹⁹, Del Ben Maria²⁰, Federici Massimo²¹, Ferri Claudio²², Fiorenza Anna Maria²³, Giaccari Andrea²⁴, Guardamagna Ornella²⁵, Iannuzzi Arcangelo²⁶, Iannuzzo Gabriella²⁷, Iughetti Lorenzo²⁸, Lupattelli Graziana²⁹, Lupi Alessandro³⁰, Mandraffino Giuseppe³¹, Marcucci Rossella³², Maroni Lorenzo³³, Mombelli Giuliana³⁴, Muntoni Sandro³⁵, Pecchioli Valerio³⁶, Pederiva Cristina³⁷, Pipolo Antonio³⁸, Pisciotta Livia³⁹, Pujia Arturo⁴⁰, Purrello Francesco⁴¹, Repetti Elena⁴², Sabbà Carlo⁴³, Sarzani Riccardo⁴⁴, Trenti Chiara⁴⁵, Vigna Giovanni Battista⁴⁶, Werba José Pablo⁴⁷, Zambon Sabina⁴⁸, Zenti Maria Grazia⁴⁹. Participant Laboratories: Bertolini Stefano³, Calandra Sebastiano⁴, Di Costanzo Alessia¹, Fortunato Giuliana⁵⁰, Spina Rossella². Collaborators: Baldera Davide⁵⁷, Banderali Giuseppe³⁷, Baratta Francesco²⁰, Beccuti Guglielmo⁸, Bertocco Sandra⁴⁸, Bruzzi Patrizia²⁸, Bucci Marco¹⁸, Buonuomo Paola Sabrina⁷, Capra Maria Elena⁹, Cardolini Iris²¹, Cefalù Angelo Baldassarre², Cinquegrani Maria³¹, Colombo Emanuela²³, Covetti Giuseppe²⁶, Cremonini Anna Laura⁵⁹, Cutolo Ada⁵⁵, D'Addato Sergio¹³, D'Ambrosio Vincenzo⁶⁰, De Corrado Giuseppe⁴², Di Pentima Chiara⁵⁶, Fimiani Fabio⁵⁴, Gentile Marco²⁷, Ghirardello Omar⁴⁶, Giusti Betti³², Grassi Davide²², Grigore Liliana⁵, Massini Giulia²⁵, Meregalli Giancarla¹⁴, Minicocci Ilenia¹, Moffa Simona²⁴, Montalcini Tiziana⁴⁰, Nascimbeni Fabio¹⁷, Negri Emanuele Alberto⁴⁵, Pavanello Chiara⁵⁸, Prati Lucia³⁶, Roscini Anna Rita²⁹, Sani Elena⁴⁹, Schaffer Alon³⁰, Scicali Roberto⁴¹, Suppressa Patrizia⁴³, Tedeschi Michele³⁸, Vinci Pierandrea¹⁰. Study Coordinating Group: Catapano Alberico Luigi⁵, Casula Manuela⁹, Gazzotti Marta⁵¹, Olmastroni Elena⁵¹, Manzato Enzo⁵², Tragni Elena⁵¹, Zampoleri Veronica⁵³.

Affiliations: ¹Dipartimento di Medicina Traslationale di Precisione, Sapienza Università di Roma - A.O. Policlinico Umberto I, Rome, Italy; ²Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza, Università degli studi di Palermo, Palermo, Italy; ³Centro Ambulatorio Dislipidemie–U.O. Clinica di Medicina Interna 1, O. Universitaria San Martino, Genua,

Italy; ⁴Laboratorio Sequenziamento Genomico, Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Modena, Italy; ⁵Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, and IRCCS Multimedica, Milan, Italy; ⁶Centro per lo Studio dell'Aterosclerosi, IRCCS Multimedica, Sesto San Giovanni, Italy; ⁷UO Malattie Rare e Genetica Medica, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy; ⁸SCDU Endocrinologia, Diabetologia e Malattie del Metabolismo, Dipartimento di Scienze Mediche, Università degli Studi di Torino, Turin, Italy; ⁹Centro Dislipidemie in Età Evolutiva, U.O. Pediatria e Neonatologia, Ospedale Guglielmo da Saliceto, Piacenza, Italy; ¹⁰S.S. Diabetologia e Malattie Metaboliche, U.C.O. Clinica Medica, ASUGI, Università di Trieste, Trieste, Italy; ¹¹Ambulatorio Dislipidemie, UO Medicina Interna, Ospedale dell'Angelo di Mestre, Venice, Italy; ¹²AOU San Luigi Gonzaga, Orbassano, Turin, Italy; ¹³U.O. di Medicina Interna Cardiovascolare, Centro Aterosclerosi, Ambulatorio Dislipidemie, IRCCS S. Orsola Ospedale Policlinico S. Orsola-Malpighi, Bologna, Italy; ¹⁴U.O.C. Malattie Endocrine and Centro regionale per il Diabete (Diabetologia), Ospedale "Treviglio-Caravaggio" di Treviglio, Bergamo, Italy; ¹⁵Ambulatorio Dislipidemie, Centro per lo Studio e la Prevenzione dell'Arteriosclerosi, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico and Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano, Milan, Italy; ¹⁶U.O.C. Cardiologia Clinica a Direzione Universitaria and U.T.I.C., A.O.R.N. "Sant'Anna e San Sebastiano", Caserta, Italy, Dipartimento di Scienze Mediche Traslazionali, Università degli studi della Campania "Luigi Vanvitelli", Naples, Italy; ¹⁷U.O. Medicina interna ad indirizzo metabolico-nutrizionistico, Centro dislipidemie e malattie metaboliche rare, Ospedale Civile Baggiovara, AOU di Modena, Modena, Italy; ¹⁸Clinica Medica, Centro di alta specializzazione per la prevenzione dell'arteriosclerosi, centro di eccellenza ESH per l'ipertensione arteriosa, centro di riferimento regionale per le Dislipidemie, Ospedale Policlinico S.S. Annunziata, Chieti, Italy; ¹⁹Centro Dislipidemie e Aterosclerosi, UOC Medicina Interna, Ospedale di Trento, Trento, Italy; ²⁰Dipartimento Scienze Cliniche, Internistiche, Anestesiologiche e Cardiovascolari—Sapienza Università, A.O. Policlinico Umberto I, Rome, Italy; ²¹Dipartimento di Medicina dei Sistemi, Università di Roma Tor Vergata, Rome, Italy; ²²Centro Ipertensione Arteriosa e Prevenzione Cardiovascolare—UOC Medicina Interna e Nefrologia, Università dell'Aquila—Dipartimento MeSVA—Ospedale San Salvatore - l'Aquila, Italy; ²³ASST-Rhodense Garbagnate Milanese, Garbagnate Milanese, Milan, Italy; ²⁴Center for Endocrine and Metabolic Diseases, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; ²⁵Paediatric Endocrinology, Department of Public Health and Paediatric Sciences, Turin University, Turin, Italy; ²⁶U.O. Medicina Interna 2, Centro per le malattie da arteriosclerosi, AORN Cardarelli, Naples, Italy; ²⁷Centro Coordinamento regionale per le Iperlipidemie, AOU Policlinico Federico II, Naples, Italy; ²⁸U.O.C. Pediatria, Azienda Ospedaliero Universitaria di Modena; ²⁹U.O. Medicina Interna Angiologia, Malattie da Arteriosclerosi, Ambulatorio di malattie del ricambio lipidico, Ospedale Santa Maria della Misericordia, Perugia, Italy; ³⁰ASL VCO - UO SOC Cardiologia, Ospedale castelli, Verbania, Italy; ³¹Department of Clinical and Experimental Medicine - Lipid Center - University Hospital G. Martino, Messina, Italy; ³²Dipartimento medicina sperimentale e clinica, Università di Firenze, AOU Careggi, Firenze; ³³Ambulatorio ipertensione dislipidemie, U.O. Medicina Generale, ASST Valle Olona, Ospedale di Gallarate, Gallarate, Italy; ³⁴Centro Dislipidemie ASST Grande Ospedale Metropolitan Niguarda, Milan, Italy; ³⁵Dipartimento di Scienze Biomediche, Università degli Studi di Cagliari and Centro per le Malattie Dismetaboliche e l'Arteriosclerosi, Associazione ME.DI.CO. Onlus Cagliari, Cagliari, Italy; ³⁶UOSD "Prevenzione cardiovascolare", Dipartimento di Scienze Mediche, Azienda Sanitaria Locale Frosinone, Frosinone, Italy; ³⁷U.O. Clinica Pediatrica, Servizio Clinico Dislipidemie per lo Studio e la Prevenzione dell'Aterosclerosi in età Pediatrica, ASST-Santi Paolo e Carlo, Milan, Italy; ³⁸AOU

San Giovanni di Dio e Ruggi d'Aragona, Salerno, Italy; ³⁹IRCCS Ospedale policlinico San Martino UOSD Dietetica e Nutrizione Clinica and Dipartimento di Medicina Interna, Università di Genova, Genoa, Italy; ⁴⁰A.O.U. Mater Domini, Catanzaro, UOC di Nutrizione Clinica, Ambulatorio Dislipidemie, Catanzaro, Italy; ⁴¹Department of Clinical and Experimental Medicine, University of Catania, Ospedale Garibaldi, Catania, Italy; ⁴²SC Diabetologia e Malattie Metaboliche, ASL AT, Asti, Italy; ⁴³U.O. di Medicina Interna e Geriatria "C. Frugoni" and Centro di Assistenza e Ricerca Malattie Rare, A.O. Universitaria Policlinico Consorziale, Università degli Studi di Bari "Aldo Moro", Bari, Italy; ⁴⁴Clinica di Medicina Interna e Geriatria, Dipartimento di Scienze Cliniche e Molecolari, Università Politecnica delle Marche e IRCCS-INRCA, Ancona, Italy; ⁴⁵Arcispedale S. Maria Nuova - Azienda ospedaliera di Reggio Emilia, Reggio Emilia, Italy; ⁴⁶U.O. Medicina Interna Universitaria, Centro per lo Studio delle Dislipidemie e dell'Aterosclerosi, Azienda Ospedaliero-Universitaria di Ferrara, Ferrara, Italy; ⁴⁷U.O. Ambulatorio Prevenzione Aterosclerosi IRCCS Cardiologico Monzino, Milan, Italy; ⁴⁸Dipartimento di Medicina, Università di Padova, Padua, Italy; ⁴⁹U.O. Endocrinologia, Diabetologia e Malattie del Metabolismo, Centro regionale specializzato per la diagnosi e terapia delle dislipidemie e aferesi terapeutica, A.O. Universitaria Integrata di Verona, Verona, Italy; ⁵⁰Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli studi di Napoli Federico II; CEINGE Biotecnologie Avanzate s.c.a.r.l., Naples, Italy; ⁵¹Centro Universitario di Epidemiologia e Farmacologia Preventiva (SEFAP), Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy; ⁵²Dipartimento di Medicina (DIMED), Sezione Geriatrica, Università di Padova, Padua, Italy; ⁵³Centro per lo Studio dell'Aterosclerosi, Ospedale E. Bassini, Cinisello Balsamo, Milan, Italy; ⁵⁴Unit of Inherited and Rare Cardiovascular Diseases, A.O.R.N. Dei Colli "V. Monaldi", Naples, Italy; ⁵⁵Dipartimento Cardio-toraco-vascolare, UOC Cardiologia, Ospedale dell'Angelo di Mestre, Venice, Italy; ⁵⁶IRCCS-INRCA, Ancona, Italy; ⁵⁷Dipartimento di Scienze Biomediche, Università degli Studi di Cagliari, Cagliari, Italy; ⁵⁸Centro Dislipidemie ASST Grande Ospedale Metropolitan Niguarda and Centro Grossi Paoletti, Dip. Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy; ⁵⁹IRCCS Ospedale policlinico San Martino UOSD Dietetica e Nutrizione Clinica, Genoa, Italy; ⁶⁰Direttore S.C. Medicina Generale, Ambulatorio ipertensione dislipidemie, U.O. Medicina Generale, ASST Valle Olona, Ospedale di Gallarate, Gallarate, Italy.

ARTICLE INFORMATION

Received September 29, 2021; accepted February 10, 2022.

Affiliations

Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy (E.O., M.G., A.L.C., M.C.); Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy (M.A.); Department of Health Promotion Sciences Maternal and Infantile Care, Internal Medicine and Medical Specialties (PROMISE), School of Medicine, University of Palermo, Palermo, Italy (M.A.); IRCCS MultiMedica, Sesto S. Giovanni (MI), Milan, Italy (A.P., A.L.C., M.C.); and Centre for the Study of Atherosclerosis, E. Bassini Hospital, Cinisello Balsamo, Milan, Italy (A.P.).

Acknowledgments

The LIPIGEN study is an initiative of the SISA Foundation supported by an unconditional research grant from Sanofi. The genetic assessment was performed in collaboration with GenInCode, Barcelona, Spain. M.G., E.O., M.C., and A.L.C. were responsible for the study concept and design. M.G. and M.C. were responsible for study management and data collection. E.O. provided methodological and statistical knowledge and performed the analysis. M.Arca, M.Averna, and A.L.C. contributed to the interpretation of the results. M.G., A.P., and M.C. wrote the article, and all authors critically revised for important intellectual content and approved the final article.

Sources of Funding

The authors received no financial support for the research, authorship, and/or publication of this article. The work of M.C. is supported by the Ministry of Health-IRCCS MultiMedica (GR-2016-02361198). The work of A.L.C. is supported by the Fondazione Cariplo (2015-0524 and 2015-0564; H2020 REPROGRAM PHC-03-2015/667837-2; ERANET ER-2017-2364981; PRIN 2017H5F943); Ministry of Health-IRCCS MultiMedica (GR-2011-02346974); SISA Lombardia, and Fondazione SISA.

Disclosures

All authors declare no support from any organization for the submitted work and no other relationships or activities that could appear to have influenced the submitted work. A.L.C. received research funding and/or honoraria for advisory boards, consultancy, or speaker bureau from Aegerion, Amgen, AstraZeneca, Eli Lilly, Genzyme, Mediolanum, Merck or MSD, Pfizer, Recordati, Rottapharm, Sanofi-Regeneron, and Sigma-Tau. M.Arca has received research grant support from Amryth Pharmaceutical, Amgen, IONIS, Akcea Therapeutics, Pfizer, and Sanofi; has served as a consultant for Amgen, Aegerion, Akcea Therapeutics, Regeneron, Sanofi, and Alfasigma; and received lecturing fees from Amgen, Amryth Pharmaceutical, Pfizer, Sanofi, and AlfaSigma. M.Averna has received grants and personal honoraria for consultancy from Aegerion, Akcea/Ionis, Alfasigma, Amgen, Amryt, Pfizer, Regeneron, and Sanofi. The remaining authors have no disclosures to report.

Supplemental Material

Figures S1–S3

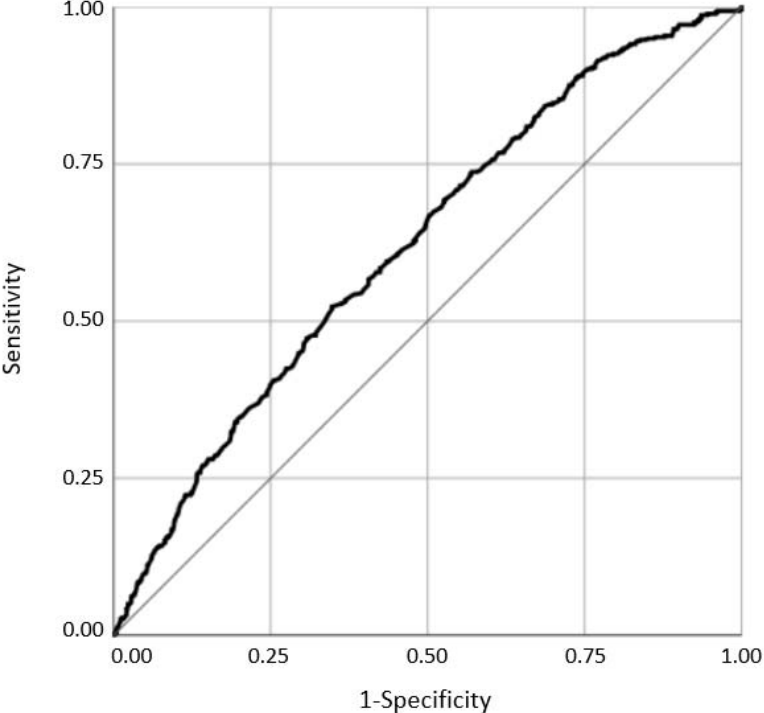
REFERENCES

- Akiyamen LE, Genest J, Shan SD, Reel RL, Albaum JM, Chu A, Tu JV. Estimating the prevalence of heterozygous familial hypercholesterolemia: a systematic review and meta-analysis. *BMJ Open*. 2017;7:e016461. doi: 10.1136/bmjopen-2017-016461
- Zamora A, Masana L, Comas-Cufi M, Vila A, Plana N, Garcia-Gil M, Alves-Cabreros L, Marrugat J, Roman I, Ramos R, et al. Familial hypercholesterolemia in a European Mediterranean population-Prevalence and clinical data from 2.5 million primary care patients. *J Clin Lipidol*. 2017;11:1013–1022. doi: 10.1016/j.jacl.2017.05.012
- Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, Wiklund O, Hegele RA, Raal FJ, Defesche JC, et al. Familial hypercholesterolemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J*. 2013;34:3478–3490. doi: 10.1093/eurheartj/ehd273
- Berberich AJ, Hegele RA. The complex molecular genetics of familial hypercholesterolemia. *Nat Rev Cardiol*. 2019;16:9–20. doi: 10.1038/s41569-018-0052-6
- Soutar AK, Naoumova RP, Traub LM. Genetics, clinical phenotype, and molecular cell biology of autosomal recessive hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 2003;23:1963–1970. doi: 10.1161/01.ATV.0000094410.66558.9A
- Natarajan P, Peloso GM, Zekavat SM, Montasser M, Ganna A, Chaffin M, Khera AV, Zhou W, Bloom JM, Engreitz JM, et al. Deep-coverage whole genome sequences and blood lipids among 16,324 individuals. *Nat Commun*. 2018;9:3391. doi: 10.1038/s41467-018-05747-8
- Sjouke B, Defesche JC, de Randamie JSE, Wiegman A, Fouchier SW, Hovingh GK. Sequencing for LIPA mutations in patients with a clinical diagnosis of familial hypercholesterolemia. *Atherosclerosis*. 2016;251:263–265. doi: 10.1016/j.atherosclerosis.2016.07.008
- Marduel M, Ouguerram K, Serre V, Bonnefont-Rousselot D, Marques-Pinheiro A, Erik Berge K, Devillers M, Luc G, Lecerc JM, Tosolini L, et al. Description of a large family with autosomal dominant hypercholesterolemia associated with the APOE p.Leu167del mutation. *Hum Mutat*. 2013;34:83–87. doi: 10.1002/humu.22215
- Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. *J Clin Endocrinol Metab*. 2012;97:3956–3964. doi: 10.1210/jc.2012-1563
- Bertolini S, Pisciotto L, Rabacchi C, Cefalu AB, Noto D, Fasano T, Signori A, Fresca R, Averna M, Calandra S. Spectrum of mutations and phenotypic expression in patients with autosomal dominant hypercholesterolemia identified in Italy. *Atherosclerosis*. 2013;227:342–348. doi: 10.1016/j.atherosclerosis.2013.01.007
- Wang J, Dron JS, Ban MR, Robinson JF, McIntyre AD, Alazzam M, Zhao PJ, Dillio AA, Cao H, Huff MW, et al. Polygenic versus monogenic causes of hypercholesterolemia ascertained clinically. *Arterioscler Thromb Vasc Biol*. 2016;36:2439–2445. doi: 10.1161/ATVBAHA.116.308027
- Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, Natarajan P, Lander ES, Lubitz SA, Ellinor PT, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet*. 2018;50:1219–1224. doi: 10.1038/s41588-018-0183-z
- Talmud PJ, Shah S, Whittall R, Futema M, Howard P, Cooper JA, Harrison SC, Li K, Drenos F, Karpe F, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolemia: a case-control study. *Lancet*. 2013;381:1293–1301. doi: 10.1016/S0140-6736(12)62127-8
- Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010;466:707–713. doi: 10.1038/nature09270
- Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. 2013;45:1274–1283. doi: 10.1038/ng.2797
- Talmud PJ, Drenos F, Shah S, Shah T, Palmieri J, Verzilli C, Gaunt TR, Pallas J, Loring R, Li K, et al. Gene-centric association signals for lipids and apolipoproteins identified via the HumanCVD BeadChip. *Am J Hum Genet*. 2009;85:628–642. doi: 10.1016/j.ajhg.2009.10.014
- Mariano C, Alves AC, Medeiros AM, Chora JR, Antunes M, Futema M, Humphries SE, Bourbon M. The familial hypercholesterolemia phenotype: monogenic familial hypercholesterolemia, polygenic hypercholesterolemia and other causes. *Clin Genet*. 2020;97:457–466. doi: 10.1111/cge.13697
- Cupido AJ, Tromp TR, Hovingh GK. The clinical applicability of polygenic risk scores for LDL-cholesterol: considerations, current evidence and future perspectives. *Curr Opin Lipidol*. 2021;32:112–116. doi: 10.1097/MOL.0000000000000741
- Averna M, Cefalu AB, Casula M, Noto D, Arca M, Bertolini S, Calandra S, Catapano AL, Tarugi P Group L. Familial hypercholesterolemia: the Italian Atherosclerosis Society Network (LIPIGEN). *Atheroscler Suppl*. 2017;29:11–16. doi: 10.1016/j.atherosclerosis.2017.07.001
- Gazzotti M, Casula M, Olmastroni E, Averna M, Arca M, Catapano AL. How registers could enhance knowledge and characterization of genetic dyslipidaemias: the experience of the LIPIGEN in Italy and of other networks for familial hypercholesterolemia. *Atheroscler Suppl*. 2020;42:e35–e40. doi: 10.1016/j.atherosclerosis.2021.01.007
- Casula M, Olmastroni E, Pirillo A, Catapano AL, Arca M, Averna M, Bertolini S, Calandra S, Catapano AL, Tarugi P, et al. Evaluation of the performance of Dutch Lipid Clinic Network score in an Italian FH population: the LIPIGEN study. *Atheroscler*. 2018;277:413–418. doi: 10.1016/j.atherosclerosis.2018.08.013
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. doi: 10.1038/gim.2015.30
- Martin AR, Kanai M, Kamatani Y, Okada Y, Neale BM, Daly MJ. Clinical use of current polygenic risk scores may exacerbate health disparities. *Nat Genet*. 2019;51:584–591. doi: 10.1038/s41588-019-0379-x
- Rieck L, Bardey F, Grenkowitz T, Bertram L, Helmuth J, Mischung C, Spranger J, Steinhagen-Thiessen E, Bobbert T, Kassner U, et al. Mutation spectrum and polygenic score in German patients with familial hypercholesterolemia. *Clin Genet*. 2020;98:457–467. doi: 10.1111/cge.13826
- Sing CF, Stengard JH, Kardya SL. Genes, environment, and cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2003;23:1190–1196. doi: 10.1161/01.ATV.0000075081.51227.86
- Mbemi A, Khanna S, Njiki S, Yedjou CG, Tchounwou PB. Impact of gene-environment interactions on cancer development. *Int J Environ Res Public Health*. 2020;17:8089. doi: 10.3390/ijerph17218089
- Tremblay J, Hamet P. Environmental and genetic contributions to diabetes. *Metab, Clin Exp*. 2019;100:153952. doi: 10.1016/j.metabol.2019.153952

28. Ottman R. Gene-environment interaction: definitions and study designs. *Prev Med.* 1996;25:764–770. doi: 10.1006/pmed.1996.0117
29. Yang Q, Khoury MJ. Evolving methods in genetic epidemiology. III. Gene-environment interaction in epidemiologic research. *Epidemiol Rev.* 1997;19:33–43. doi: 10.1093/oxfordjournals.epirev.a017944
30. Flowers E, Froelicher ES, Aouizerat BE. Gene-environment interactions in cardiovascular disease. *Eur J Cardiovasc Nurs.* 2012;11:472–478. doi: 10.1016/j.ejcnurse.2011.06.001
31. Lewis CM, Vassos E. Polygenic risk scores: from research tools to clinical instruments. *Genome Med.* 2020;12:44. doi: 10.1186/s13073-020-00742-5
32. Trinder M, Francis GA, Brunham LR. Association of monogenic vs polygenic hypercholesterolemia with risk of atherosclerotic cardiovascular disease. *JAMA Cardiol.* 2020;5:390–399. doi: 10.1001/jamacardio.2019.5954
33. D'Erasmus L, Minicocci I, Di Costanzo A, Pigna G, Commodari D, Ceci F, Montali A, Brancato F, Stanca I, Nicolucci A, et al. Clinical implications of monogenic versus polygenic hypercholesterolemia: long-term response to treatment, coronary atherosclerosis burden, and cardiovascular events. *J Am Heart Assoc.* 2021;10:e018932. doi: 10.1161/JAHA.120.018932
34. Knowles JW, Rader DJ, Khoury MJ. Cascade screening for familial hypercholesterolemia and the use of genetic testing. *JAMA.* 2017;318:381–382. doi: 10.1001/jama.2017.8543
35. Sharifi M, Higginson E, Bos S, Gallivan A, Harvey D, Li KW, Abeysekera A, Haddon A, Ashby H, Shipman KE, et al. Greater preclinical atherosclerosis in treated monogenic familial hypercholesterolemia vs. polygenic hypercholesterolemia. *Atherosclerosis.* 2017;263:405–411. doi: 10.1016/j.atherosclerosis.2017.05.015
36. Umans-Eckenhausen MA, Defesche JC, van Dam MJ, Kastelein JJ. Long-term compliance with lipid-lowering medication after genetic screening for familial hypercholesterolemia. *Arch Intern Med.* 2003;163:65–68. doi: 10.1001/archinte.163.1.65

SUPPLEMENTAL MATERIAL

Figure S1. Receiver operating characteristic (ROC) curve for LDLc-score for the diagnosis of a polygenic aetiology (A) and classification of adults with (FH/M+) and without (FH/M-) according to a 0.905 cut off in LDLc-score (B).



Model (AUC): 0.59 [0.56-0.62]
 Cut-off: 0.905
 Sensitivity: 77%
 Specificity: 36%
 Youden index: 0.14

	FH/M-	FH/M+	total
≤0.905	148	315	463
	31.97	68.03	
	22.98	36.00	
>0.905	496	560	1056
	46.97	53.03	
	77.02	64.00	
Total	644	875	1519

(A)

(B)

Figure S2. Correlation between LDL-C levels and LDLc-score in mutation-positive FH subjects carrying the mutation c.662A>G (p.Asp221Gly).

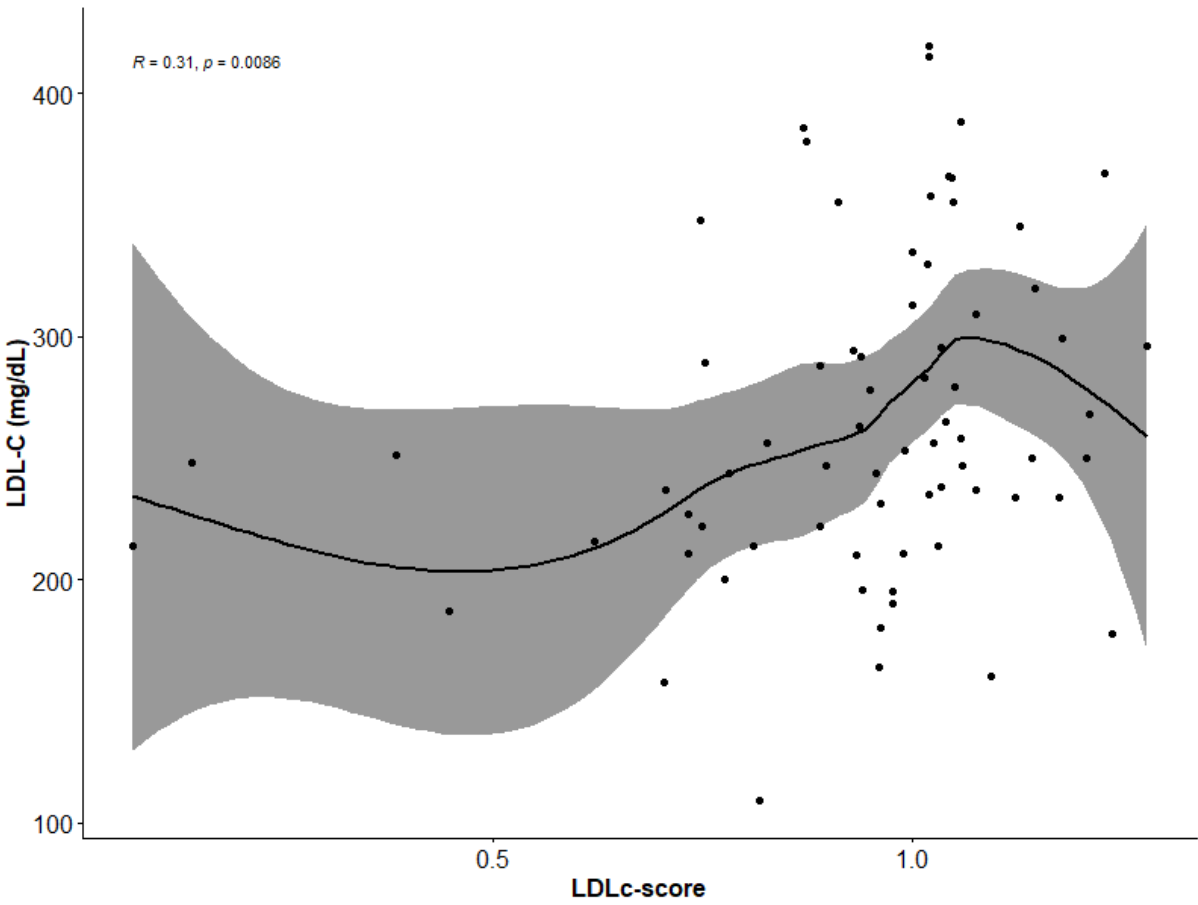


Figure S3. LDL-C levels by LDLc-score deciles (as defined by Talmud et al.¹³) in UK Whitehall II study and in the FH/M- and FH/M+ LIPIGEN cohorts.

